REMARKS

Entry of the foregoing, reexamination and reconsideration of the subject application, as amended, pursuant to and consistent with 37 C.F.R. § 1.112, are respectfully requested in light of the remarks which follow.

I. Amendments to the Specification

By the foregoing amendments to the specification, the specification has been amended to remove hyperlinks, insert sequence identifiers, and replace "Sequence ID No." and other improper terminology with "SEQ ID NO:" (*see below* for more detail). No new matter has been added.

II. Amendments to the Claims

By the foregoing amendments to the claims, claims 1-7 and 9-26 have been amended, and claim 8 has been canceled.

In particular, claim 1 has been amended to recite that the domain of the toxic membrane protein is a transmembrane domain. Support for this amendment can be found at least at page 7, lines 17-21 of the specification.

In addition, claim 1 has been further amended to recite the subject matter of claim 8.

Claim 9 has been amended to depend from claim 1 rather than from claim 8.

Other amendments to the claims have also been made to clarify the claim language, for consistency, and to bring the claims into better conformance with U.S. patent practice. These amendments are merely editorial in nature and are not intended to change the scope of the claims or any elements recited therein.

The amendments to the claims, including cancellation of claims, have been made without prejudice or disclaimer to any subject matter recited or canceled herein. Applicants reserve the right to file one or more continuation and/or divisional applications directed to any canceled subject matter. No new matter has been added, and entry of the foregoing amendments to the above-identified application are respectfully requested.

III. Response to Objections to the Claims

A. At page 3 of the Office Action, claims 5, 6, 10, 11, 16 and 23-25 have been objected to for including improper sequence identifiers.

The sequence identifiers have been corrected to "SEQ ID NO:" throughout the claims.

B. Also at page 3, claims 10, 11, 16 and 23-25 have been further objected to for depending from a rejected claim (*i.e.* claim 1).

In response, Applicants hereby traverse the outstanding rejections to claim 1 (*see below* for more detail).

In view of the above, Applicants respectfully request reconsideration and withdrawal of the objections to the claims.

IV. Response to Objections to the Specification

At page 4 of the Office Action, the specification has been objected to for the following reasons:

A. The Examiner has objected to the specification for referring to sequences without also identifying them by the sequence identifier assigned to them in the Sequence Listing (citing Figure 1 of the Drawings).

In response, Applicants have amended the "Brief Description of the Figures" section of the specification to identify the sequences shown in the figures by the sequence identifier assigned to the sequences in the Sequence Listing. In particular, with regard to the peptide sequence "TME2" in Figure 1, this sequence has been appropriately identified as "amino acids 2-31 of SEQ ID NO: 2."

B. The Examiner has also objected to the specification because it contains embedded hyperlinks and/or other form of browser-executable code at page 19.

Applicants have further amended the specification by deleting the hyperlinks at page 19.

In view of the above, Applicants respectfully request reconsideration and withdrawal of the objections to the specification.

V. Response to Claim Rejections Under 35 U.S.C. § 102

A. At pages 4-5 of the Office Action, claims 1-3, 12, 17-22 have been rejected under 35 U.S.C. § 102(b) as purportedly being anticipated by Bolling et al. (U.S. Patent No. 5,322,769) as evidenced by Weiner et al. (U.S. Patent No. 6,881,558 B1). This rejection is respectfully traversed.

To expedite prosecution in the present application, and not to acquiesce to the

Examiner's rejection, the claims have been amended as described above. In particular, claim 1 has been amended to recite that the "domain of a toxic membrane protein" is a "transmembrane domain."

Bolling et al. describe in Example 5 (in view of Example 4) a fusion protein comprising CKS, a linker region including an AspPro (*i.e.* DP) peptide, and a portion of a synthetic p41 gene. The synthetic p41 gene component (synp41d) encodes a deletion mutant of the gp41 protein which contains a 38aa hydrophobic region deletion from A1a674 to Va1711 (based on gp160 numbering).

The envelope glycoprotein gp160 (envelope protein) of the HIV virus (reference SWISS-PROT 070902) contains:

- a surface protein: SU, glycoprotein 120 (gp120), and
- a transmembrane protein: TM glycoprotein 41 (gp41).

Accordingly, the synp4ld of Bolling et al. does not comprise the transmembrane region (TM) of the transmembrane protein of HIV. Therefore, Bolling et al. do not describe an expression system comprising a nucleotide sequence encoding a toxic membrane protein or a transmembrane domain of a toxic membrane protein.

Because each and every element of the claimed invention is not taught in Bolling et al., Applicants respectfully request reconsideration and withdrawal of this rejection.

B. At pages 5-6 of the Office Action, claims 1-3, 12 and 17-22 have been rejected under 35 U.S.C. § 102(b) as being anticipated by Chan et al. (EP 0 212 532; published 1987) as evidenced by Weiner et al. (U.S. Patent No. 6,881,558 B1). This rejection is respectfully traversed.

To expedite prosecution in the present application, and not to acquiesce to the Examiner's rejection, claim 1 has also been amended to recite that the expression system comprises a "nucleotide sequence encoding a soluble protein" upstream of the "nucleotide sequence encoding the dipeptide Asp-Pro."

Chan et al. disclose an expression system comprising successively, in the 5'-3' direction, a nucleotide sequence encoding TrPLE, a linker encoding the DP peptide, and the ENV gene of the AIDS virus or a portion thereof which preferably encodes the polypeptide KAL-10.

The expression system disclosed in Chan et al. does not include a nucleotide sequence encoding a soluble protein upstream of the nucleotide sequence encoding the dipeptide Asp-Pro,

as recited in the present claims.

Because each and every element of the claimed invention is not taught in Chan et al., Applicants respectfully request reconsideration and withdrawal of this rejection.

VI. Response to Claim Rejections Under 35 U.S.C. § 103

A. At pages 6-8 of the Office Action, claims 4-7 have been rejected under 35 U.S.C. § 103(a) as purportedly being unpatentable over Bolling et al. (U.S. Patent No. 5,322,769) as applied to claim 1 and further in view of De Beeck et al. (Journal of Biological Chemistry, 2000, Vol. 275, p. 31428-31437, in IDS of 6/23/05) and Arechaga et al. (FEBS, 2000, Vol. 482, p. 215-219) as evidenced by Caccaglione et al. (Virus Genes, 2000, Vol. 21, p. 223-226, in IDS of 6/23/05).

B. At pages 8-10 of the Office Action, claims 8, 9 and 15 have been rejected under 35 U.S.C. § 103(a) as purportedly being unpatentable over Bolling et al. (U.S. Patent No. 5,322,769) in view of De Beeck et al. (Journal of Biological Chemistry, 2000, Vol. 275, p. 31428-31437, in IDS of 6/23/05) and Arechaga et al. (FEBS, 2000, Vol. 482, p. 215-219) as evidenced by Caccaglione et al. (Virus Genes, 2000, Vol. 21, p. 223-226, in IDS of 6/23/05) as applied to claim 1 and further in view of Smith et al. (Gene, 1988, Vol. 67, p. 31-40) and Fiaschi et al. (FEBS, 1995, Vol. 367, p. 145-148).

For at least the reasons set forth above, Bolling et al. do not teach or even suggest an expression system comprising a nucleotide sequence encoding a toxic membrane protein or a transmembrane domain of a toxic membrane protein. Applicants respectfully submit that the secondary references cited by the Examiner fail to remedy the serious deficiencies of Bolling et al.

In particular, De Beek et al. describe the role played by the transmembrane domains (TMDs) in the mechanism of heterodimerization of Hepatitis C virus envelope proteins El and E2. However, the reference does teach or suggest the claimed system for expressing toxic proteins or membrane domains thereof.

Furthermore, Arechaga et al. describe the use of mutant host strains selected from *E. coli* BL21(DE3) that allow over-production of subunit b from the Fo membrane sector of *E. coli* ATP synthase that could not be expressed in BL21(DE3). However, the reference does not teach or suggest the claimed system for expressing toxic membrane proteins or membrane domains thereof.

In addition, Ciccaglione et al. describe the use of the BL21(DE3)pLysS strain for expressing Hepatitis C virus El protein that could not be expressed in BL2(DE3) due to its toxicity. However, this reference does not teach or suggest the system claimed herein for expressing toxic membrane proteins or membrane domains thereof.

Furthermore, Smith and Johnson describe the use of a pGEX plasmid based on the enzyme glutathione GST to allow expression of various eukariotic polypeptides in *E. coli* as soluble and stable GST fusion proteins which can be readily purified. However, Smith and Johnson do not tackle the technical problem of the toxic effect of membrane proteins to be expressed for the host. Moreover, the present inventors have shown that the use of GST fusion proteins (GST-TME 1 or -TME2), in contrast to the present invention, do not resolve the technical problems related to toxicity. Accordingly, Smith and Johnson do not teach or suggest using the claimed system for expressing toxic membrane proteins or membrane domains thereof, and based on the cited references a person of ordinary skill in the art would not have predicted the suppression of the toxic effect as discovered by the present inventors. The finding that toxicity of the protein for the host is more effectively suppressed when the expression system further comprises a nucleoide sequence encoding a soluble protein upstream of the DP sequence was particularly surprising.

Finally, Fiaschi et al. describe cloning and exprssion of the cDNA encoding the erythrocyte isoenzyme of human acylphosphate using a pGEX-KT vector. The reference does not relate to the technical problem (the toxic effect of expressed membrane proteins on the host) solved by the present invention. Moreover, the present inventors have shown that the use of GST fusion proteins (GSTTME1 or -TME2) does not resolve such technical problems. Thus, Fiaschi et al. do not teach or suggest using the claimed system for expressing toxic membrane proteins or membrane domains thereof, and in addition do not teach or suggest that the suppression of the toxic effect of a protein for the host would be more effective when the expression system comprises a nucleoide sequence encoding a soluble protein upstream of the DP sequence.

The combination of the references cited by the Examiner would not have suggested the particular combination of elements required by the present claims to a person of ordinary skill in the art at the time that the invention was made. In particular, even by combining the secondary references cited by the Examiner, a person of ordinary skill in the art would not

have obtain the claimed invention starting from the expression system of Bolling et al. which always uses the CKS fusion protein.

Furthermore, by combining Bolling et al., De Beek et al., and Arechaga et al. as evidenced by Ciccaglione, one of skill in the art would have not been motivated to express De Beek et al.'s toxic HCV TME2 membrane domain in Bolling et al.'s bacterial expression vector (comprising in the 5'-3' direction a nucleotide sequence encoding CKS, and a linker region including a DP peptide), because Bolling et al. has only demonstrated successful expression of synp41d, which does not contain the transmembrane domain of the envelope protein. The same reasoning applies for the combination of Bolling et al., De Beek et al., and Arechaga et al. as evidenced by Ciccaglione et al., Smith et al. and Fiashi et al.

A specific understanding or principle within the knowledge of a skilled artisan that would have motivated one to make the combination in the manner claimed is required to make out a proper *prima facie* case of obviousness. *In re Kotzab*, 217 F.3d 1365, 1369-70, 55 U.S.P.Q.2d 1313, 1318 (Fed. Cir. 2000). In other words, the Examiner must provide a logical reason as disclosed in the prior art at the time of the invention for combining the references so as to arrive at the invention. Otherwise, the use of such teachings as evidence of obviousness must be considered impermissible hindsight. *See, e.g.*, *In re Nomiya*, 509 F.2d 566, 184 U.S.P.Q. 607 (CCPA 1975); *Ex parte Stauber*, 208 U.S.P.Q. 945, 946 (Bd. Pat. App. & Intf. 1980).

Applicants note that the Examiner was compelled to combine four or six references in order to issue the rejections under 35 U.S.C. § 103. Moreover, as discussed above many of the references do not deal with the problem of host toxicity related to the expression of membrane proteins. The Examiner should bear in mind that the documents produced in the search have, of necessity, been obtained with foreknowledge of what matter constitutes the present invention. The cited documents have clearly been chosen by the Examiner using *ex post-facto* analysis.

The Office has relied upon generalizations and conclusory statements regarding the purported motivation provided by the cited art. In these general statements, the Office has failed to adduce specific reasons that one of ordinary skill in the art would have had to make the specific invention now claimed. Therefore, these statements are not sufficient to meet the Office's burden of presenting a logical reason for one of ordinary skill in the art to make the specific invention that is now. Rejections on obviousness grounds cannot be sustained by

mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness. *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. ___, 127 S.Ct. 1727, 1739, 82 U.S.P.Q.2d 1385, 1396 (April 30, 2007) (citing *In re Kahn*, 441 F.3d 977, 988, 78 U.S.P.Q.2d 1329 (Fed. Cir. 2006)).

For at least the foregoing reasons, withdrawal of the obviousness rejections is respectfully requested.

CONCLUSION

This response is made without prejudice or disclaimer to any non-elected subject matter, and Applicants reserve the right to file one or more continuation and/or divisional applications directed to any non-elected subject matter.

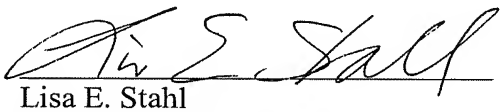
In view of the foregoing, further and favorable action in the form of a Notice of Allowance is believed to be next in order. Such action is earnestly solicited.

In the event that there are any questions related to this response, or the application in general, it would be appreciated if the Examiner would telephone the undersigned attorney at the below-listed telephone number concerning such questions so that prosecution of this application may be expedited.

Respectfully submitted,

BUCHANAN INGERSOLL & ROONEY PC

Date: May 28, 2008

By: 
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